XLIX.—*On the Developmental History of the Nematode Worms.* By RUDOLPH LEUCKART.

[Continued from p. 347.]

I AM acquainted with phases of development similar to those last described in *Sclerostomum equinum,* the notorious palisado worm of the horse (*Strongylus armatus,* auctt.), not, however, from the intestine, in which I have always found the worm in full sexual maturity, but from aneurismatic dilatations of the mesenteric arteries, which are produced by the parasitism of this animal, and frequently (when great numbers are present) increase to a very considerable size. Contrary to what occurs in the intestine, we find in these aneurisms nothing but young forms of the worm (the so-called small variety), from which I am inclined to think that the worm passes only thence into the intestine, probably through the peripheral ramifications of the mesenteric arteries. If my supposition be well founded, the worm must certainly bore through the wall of the intestine at a time when it is already of the considerable length of 15–20 millims. (thickness = 1 millim.); but the powerful armature of the mouth, and especially the denticulation of the margins of the lips, which almost involuntarily remind one of a trephine, show that this process cannot be attended with any great difficulties.

The youngest of the worms detected by me (as also previously by Mehlis, Gurlt, and Dujardin) in the aneurisms had a length of about 10–12 millims. From their grade of development, they might be compared with the intermediate forms of *Dochmius* and *Cucullanus,* although differing from these not only by their larger size, but also by the want of the buccal cup. Instead of the latter, the worms (like the young forms of *Ollulanus*) possess in the periphery of the gaping buccal orifice a rosette-like plate with six laminæ, which exhibit an elegant sculpture. The caudal extremity is short and pointed, and, in the small specimens, of perfectly concordant structure; whilst among the larger worms we may distinguish specimens with slenderer tails, and others with a shorter and plumper posterior extremity. In the former the tail is longer than broad; in the latter, on the contrary, broader than long, and truncated at the apex (which is directed dorsally).

If we subject the latter specimens to a closer examination, we at once find that they are the male animals. However, we are led to this conviction less by the still rudimentary internal sexual organs than by the copulatory apparatus, which is developed beneath the cuticular covering of the inflated posterior extremity, and becomes more and more recognizable with increasing age.

The formation of these organs is commenced by the parenchyma of the body in the periphery of the anus separating from the chitinous envelopes and gradually removing further from it. In this way a dome-shaped cavity is produced in the interior of the caudal extremity, its axis being traversed by the lobately projecting rectum. Of course, the dome is not perfectly symmetrical, but is obliquely truncated at its lower extremity in correspondence with the structure of the tail, with a shorter ventral and a longer dorsal lateral margin.

As soon as the walls of the dome have been enlarged so as to produce a certain amount of surface, their parenchyma begins to undergo unequal division in a definite manner. There are produced numerous radiating blastema-streaks, which are united to each other by a more lamellar diffusion,—in other words, the ribs of the caudal hood, with their uniting membranes, structures which consequently correspond morphologically with the previous caudal extremity, and no doubt have the same origin essentially also in *Dochmius* and the other Strongylidæ.

But during the formation and development of the caudal hood a peculiar transformation has also taken place at the anterior extremity of our worm, the future buccal cup having presented its first traces here, especially in the periphery of the anterior extremity of the œsophagus. The developmental history of this apparatus agrees in its essential particulars with the processes already described in *Cucullanus,* except that, in consequence of the much larger size of the worm, they are much more striking and may be more distinctly traced. To this may be added the circumstance that the buccal cup of *Sclerostomum* is composed of several (four) different portions, lying one behind the other like segments, so that it has a more complicated structure than in *Cucullanus.* Of course this circumstance has an influence on the development. It would, however, lead me too far if I were to attempt a detailed description of the different developmental processes; consequently I shall only remark that the segments of the buccal cup very early rise like so many terraces on the inner wall of the new buccal cavity. As this takes place at a period when the œsophagus is still situated close behind the buccal rosette, it follows that at their first appearance these rings occupy precisely a reversed position; that is to say, the finally last segment is at first the foremost one. A change of position commences only at the retrogression of the œsophagus, which gradually brings the buccal cup to its definitive structure.

The first embryonic form of *Sclerostomum equinum* is still unknown to me; but I can hardly doubt that it is essentially constructed as in *Dochmius,* and also leads the same life. I am less doubtful about this, because in *S. hypostomum* of the sheep*, the eggs of which pass out in a state of segmentation, I observed *Rhabditis*-like young in the course of a few days, and these differed from the embryos of *Dochmius trigonocephalus* only by a somewhat larger size (0·46 millim.) and an extraordinarily long, subulate caudal extremity (of fully 0·15 millim.). The animals lived in mud for several weeks, but only increased a little in size (to 0·53 millim.), and then, towards the end of the third week, underwent a change of skin, in which the tail was lost. As the dental apparatus of the posterior pharyngeal bulb had previously disappeared, and the two dilatations were not very prominent, the embryo at this period of development (length = 0·46 millim., of which only 0·04 was due to the abbreviated tail) had a great resemblance to the embryos of *Strongylus filaria*. Unfortunately I did not succeed in observing the further metamorphoses. The worms remained unaltered in water, and gradually began to die off; so that I was induced to administer the remainder of them at two different times (on the 20th and 27th of February) to a young sheep; but on dissection (on the 6th of March), I could not find any trace of them. It is therefore possible that the life-history of our *Sclerostomum* is more complicated than that of *Dochmius*, and that its free existence is not followed immediately by parasitism in its definitive host.

The Strongylidæ with a buccal armature are not, however, the only Nematoda with Rhabditiform embryos. The same young forms recur in other species, even systematically distant—for example, in *Ascaris acuminata* of the frog, the embryos of which, as is well known, are developed in the body of their mother, and have been kept alive for a long time in water by Göze and Dujardin†. On close examination, we recognize in these embryos the *Rhabditis*-form above described, with teeth in the posterior pharyngeal dilatation, and a pointed but short tail. The length of the little worm is 0·6 millim., and its thickness 0·035 millim.; the embryo has consequently a tolerably thick form. But this appearance is altered in the course of a few days. The embryo grows, and, indeed, so rapidly that in a week the length of its body has increased almost threefold (nearly 1·5 millim.). But

* It may be mentioned, in passing, that this *Sclerostomum*, which inhabits the colon, belongs, like *Oxyuris curvula*, to the *dung-feeding* Nematoda. In the buccal cup and intestine we constantly find numerous vegetable particles derived from the vicinity; these form black excrements, which accumulate at the anal orifice, and in the females form an incrustation surrounding the whole abdominal extremity like a hood.

† The same is stated by Spencer Cobbold of *Ascaris osculata* and *A megalocephala*, but probably, as regards the latter, incorrectly; for I have preserved its eggs, with the embryos contained in their interior, unchanged in water for two years.

it is only the length that increases. The transverse section of the body remains almost unchanged, and thus the worm gradually alters its original form for a more slender one, at the same time increasing in mobility in the same proportion. The pharynx and tail retain their previous form, and undergo but little alteration in size, whilst in the periphery of the buccal orifice three small papillæ gradually sprout forth, and a few (usually four) oil-drops accumulate in the body-cavity on each side behind the anterior pharyngeal dilatation, and, remaining pretty regularly grouped, produce almost the impression of eyes destitute of pigment.

In this state I have sometimes met with the young worms in the nasal cavity and rectum of the frog; so that one might be led to suppose that they may be converted, after immigration, directly into the definitive form. The experiments made by me in this direction, however, produced no results. Once, certainly, the young worms were found still unchanged in the rectum on the sixth day after their transfer; but otherwise they seem generally to die pretty quickly.

The example of *Ascaris acuminata* shows us, even more strikingly than that of *Dochmius*, that the free young states of the Nematoda not unfrequently attain a high degree of independency. But *A. acuminata* by no means reaches the final limit of this development. There are Nematoda *the embryos of which even attain sexual maturity in their* Rhabditis-*form, and only become parasitic again in their progeny*—Nematoda, consequently, the history of which presents us with no simple alternation of the conditions of life, but with an alternate sequence of free and parasitic generations. And, what is most wonderful, *both these generations are sexually developed*—both are produced from ova. Here, therefore, we have nothing to do with an ordinary alternation of generations, such as occurs, for example, in the *Distomeæ*, but with a process hitherto almost unheard of in the animal kingdom, and which calls for our consideration the more, because we are accustomed to regard the sexual development of an animal not merely as the sign of its perfect maturity, but also as the criterion of specific individuality.

The roundworm which undergoes this peculiar development is one which has been repeatedly investigated—the well-known *Ascaris nigrovenosa* of the lungs of our brown frog (*Rana temporaria*).

The embryos of this Nematode worm*, as everyone knows,

* In the investigation of the life-history of *Ascaris nigrovenosa* I was gratified by the participation of a talented young zoologist, M. Elias Mecznikow, of Charkow, who has also taken a lively interest in my other observations and experiments upon Nematoda. With regard to the pre-

are developed in the body of the mother like those of *Ascaris acuminata*, and usually pass into the stomach of the host still in the egg-capsule, in order there, after boring through the latter, to collect gradually in many hundreds in the rectum, in the form of small rapidly-moving vermicles; they present in their external structure a great agreement with the Rhabditiform young states of other species. They have a rather stout form, and a length of 0·4 millim. The tail is short and pointed, and the anterior, buccal extremity is furnished with three small cuticular papillæ, which I have not met with elsewhere among the allied forms. The structure of the commencement of the genital organs is still more divergent. Whilst this is elsewhere (except in the *Trichinæ*, the embryos of which are peculiar in many other respects) always developed in the form of a small and nearly homogeneous clear corpuscle (0·02 millim. in length, with usually a simple nucleus), which shimmers through the ventral wall of the embryos about the middle of the chyle-intestine, it appears in the youngest forms of *A. nigrovenosa* as a considerable sac, of 0·08 millim. in length and 0·012 millim. in thickness, enclosing numerous distinctly recognizable cells with vesiculiform nucleus and nucleolus (cell = 0·007 millim., nucleus = 0·0043 millim.). In previous grades of development this sac is recognized as an aggregation of embryonal cells, which separates from the other elements of the body of the embryo, and only changes in this respect, that the cells lose their previous coarsely granular texture, and acquire a more transparent appearance.

When the embryos are removed from the rectum of the frog (or even from the body of the mother) and kept in moist earth, they begin not only to grow rapidly, but also to develope their sexual organs; so that in a short time we have, instead of the previous young forms, sexually mature male and female animals. The duration of this period of development depends on external circumstances, especially the surrounding temperature. In the height of summer a single day sometimes suffices, whilst in winter it is a week or more (in the case of embryos taken from the body of the mother even as much as twelve days) before the animals arrive at sexual maturity.

The sexual differentiation commences before the middle of this developmental period. It is introduced by a change of skin, after which the male individuals are to be distinguished by the shorter and blunter form of the caudal extremity, from the

---

tensions raised by him in connexion with these investigations (Müller's Archiv, 1865, Heft 4) I refer to the next part of that journal, and the communication published in it by me.

otherwise still perfectly concordant females. At this time the genital sac measures about 0·15 millim., or more than half the length of the chyle-intestine, which, like all the rest of the body, has hitherto increased but little in length. After the change of skin, the growth advances far more rapidly, with increasing sexual development, especially in the female individuals, which, at the time of copulation, are about 0·65 millim. in length, while the males scarcely measure more than 0·50 millim. The transverse diameter also is pretty considerable, still especially in the females (at the level of the genital orifice, about the middle of the intestine = 0·05, in the males only 0·037 millim.), so that the form of our animals cannot well be denominated slender. In accordance with this, the movements in general are slow and clumsy.

In the female apparatus we distinguish, besides the two ovaries, which run through almost the whole length beneath the intestine, and the short vagina with its thin chitinous tube seated perpendicularly upon the outer integuments, also a middle section of moderate length, the walls of which possess a distinctly cellular structure. From its position, this section represents the so-called uterus of other Nematoda; but this denomination is perhaps not very applicable in this case, although in pregnant individuals it assists in the reception of the young. Before the development of the latter it is very distinctly and sharply marked off from the extremely thin-walled ovarian tubes, the contents of which are formed, throughout their whole length, of coarsely granular ova. In the blind extremities of the tubes, which are sometimes extended and sometimes bent in the form of horns, the ova are but small. But this is not the case at their commencement, where, at the time of copulation, we find ova fully 0·04 millim. in length (transverse diameter 0·013, germinal vesicle 0·008, germinal spot 0·0016 millim.). The number of these large ova is, however, but small, rarely exceeding four (in winter scarcely ever more than two).

The male apparatus, as usual, consists of a single testis, which runs backward beneath the intestine in the form of a thin-walled, usually simply extended tube, and, before its union with the end of the intestine, is produced into a short and muscular vas deferens. Beside the cloaca there are two short lanceolate spicula, which, with a third smaller chitinous piece, function as the copulatory organ. All three are produced by local development from the originally quite simple chitinous tube of the extremity of the intestine, as is also observed to be the case in other Nematoda. The short caudal point of the male is incurved and beset with a few small papillæ to the right and left of the median line. The extreme end bears a larger cylindrical papilla.

The seminal elements have retained their early cellular form in the upper extremity of the testis. Posteriorly these cells increase, with simultaneous formation of granules, to balls of fully 0·02 millim., which break up by quadripartition, and then form the genuine seminal corpuscles (of 0·005 millim.) not unfrequently met with after copulation, even in the interior of the female organ among the ova.

After impregnation the eggs continually increase in size (up to 0·08 millim. in length and 0·04 millim. in thickness); and at the same time commences the ordinary segmentation, which soon (in summer sometimes on the third day) leads to the separation of a long and slender embryo of disproportionate size (0·25 millim. in length). The number of these embryos of course depends upon that of the mature ova; and this, as already remarked, is in summer nearly twice as great as in winter. However, it is not only the ova (even the immature ones) that increase in size after impregnation, but also the female animals, which in the gravid condition (although only in summer) grow to a length of 1 millim.

Originally, of course, the embryos lie within the genital tube, enclosed in a thin egg-membrane. But the latter is lost as soon as the rolled-up embryo begins to extend itself. The delicate wall of the genital tube also can only resist the movements of the embryo for a short time. Soon after their development the young are seen free in the body-cavity of the mother, the wall of the genital tube being destroyed, and the mass of eggs scattered through the whole body.

This destruction of the genital tube is, however, only the introduction to a further breaking-up, which does not continue confined to the ova, but soon attacks the chyle-intestine, and finally even the pharynx and the muscular mass of the body. All these structures break up, under the constant lively movements of the embryos, into a finely granular detritus. In four or five days after the commencement of the experiments (at least in summer, for in winter this period is extended to ten or twelve days) there remains nothing of the original worm except the external chitinous envelope, with the embryos, whose undulatory movements are so strong that, at the first glance, the mother might be supposed to be still alive.

As long as the embryos remain in the body of their mother they are, as regards the structure of the pharynx, regular Rhabditides, with two dilatations, and teeth in the posterior bulb. But hardly have they escaped from their surrounding membrane than both the teeth and the dilatations are lost. The pharynx then forms a slender cylinder, with a slight thickening at the hinder extremity. The animals are also remarkably different from their parents in other respects. They have a more slender form (length=0·5–0·6, thickness=0·02–0·023 millim.) and an extraordinary mobility. The tail bears a small stiff point. The cuticle is distinctly longitudinally striated, and the genital rudiments in the interior are of small size.

In this state the young worms remain for a long time, perhaps for weeks, without any alteration. They live both in mud and water, and also occasionally penetrate into the mollusks (*Physæ* and *Paludinæ*) which live in company with them. Apparently they select the mouth as the starting-point of their wanderings; at least I have repeatedly met with them in the intestines of these animals, as well as in the body-cavity. In the latter situation the worms have cast their former cuticle and the caudal point, and likewise somewhat changed the form of the head.

This immigration into snails is, however, by no means absolutely necessary for our worms. The young worms may also be converted into the known form of *Ascaris nigrovenosa* by direct transfer to the frog.

The method employed by me in these experiments was as follows. I placed the earth inhabited by the little worms in the throat of the frog, and spread it out as much as possible with the handle of the scalpel. Direct transfer into the lungs (through the glottis and penetrating skin-wounds) did not answer. The frogs certainly survived the operation; but the change occurring in consequence of injection in the lungs (strong congestion) acted so injuriously on the parasites, that they could never be found a few days afterwards. However, I will not assert that the method above recommended led to the desired result in all cases. As the frogs soon swallow the introduced earth, most of the young worms get into the stomach of the animal experimented on, where they are certainly to be met with alive for one or two days; but finally they all die without undergoing any essential alteration. Never more than a few specimens penetrated (through the glottis) into the lungs—at the utmost eight to ten, often only a single one, and not unfrequently none at all.

I presume that the worms usually immigrate into the throat of the frog by their own motory powers. As they live in moist earth, we might also in this way explain the fact that *Ascaris nigrovenosa* is more frequently met with in the land-frog (*Rana temporaria*) than in the water-frog (*R. esculenta*), although the latter is as available as the former for the helminthological experiment.

The first changes of the immigrated worms (even of those which passed into the stomach) consist in the change of skin already mentioned. Within twelve hours after transfer the worms are seen with an obtuse tail. In the periphery of the buccal orifice there are three small lip-like processes, enclosing a small, almost spherical buccal cavity, with a strong chitinous wall. In the following days the size of the body increases considerably. At the end of the first week, the worms not unfrequently measure as much as 1 millim.; in the second week they increase to 3·5 millims. All the individuals do not, however, grow equally fast; among the larger ones we occasionally find small specimens measuring scarcely two-thirds of the former, as, indeed, is not unfrequently observed in helminthological experiments. The increase in thickness at first pretty nearly keeps pace with that of the length. At 0·85 millim. it is about 0·04, and at 2 millim. about 0·09. But when the worm has attained the latter size, and the intestine, previously slightly coloured by the browning of the epithelial cells, begins to be filled with blood, the transverse diameter of the young worms increases considerably, so that the original slender form gradually gives place to a shorter and stouter one. Worms of 3·5 millims. are fully 0·16 millim. in thickness, and those of 5 millims. in length (such as are met with towards the end of the third week) are as much as 0·23 millim. thick. The extreme caudal point alone takes no part in this thickening. It remains thin and slender, as in the worms of the first week, and then, as might be expected, is very sharply marked off from the rest of the body, almost in the form of a spine.

This increase of size of the young *A. nigrovenosa* does not, however, take place without repeated sheddings of the cuticle. This is most distinct in the later stages, where the swelling body is usually surrounded by the remains of the cast-off and wrinkled skin, as if by a scaly covering. Of course, also, the cuticle constantly becomes thicker with the increase of the size of the body, and the musculature makes its appearance more and more distinctly.

The differentiation of the sexual organs commences even before the length of the body has reached 1 millim. About this time the vagina may first be perceived, a little behind the middle of the body, with two horns running from it forward and backwards, which are at first short and thin, and terminate cæcally at a distance of about 0·07 millim. from the genital orifice. In individuals of 2 millims. in length each of the two genital tubes measures about 0·3 millim. Their course is very irregular, so that the end is scarcely 0·2 millim. from the orifice. The inferior section of the tube is the thickest (0·025 millim.), and is distinguished by an internal epithelial coat. A little further up we see a portion with delicate annular fibres; and this is followed by the longest and thinnest section, the true ovary, in the cæcal extremity of which we may already detect distinct ova, of 0·01 millim. in diameter (germinal vesicle = 0·007 millim.). In worms of 3·5 millims. these ova have attained nearly their full size (length = 0·08, thickness = 0·035, germinal vesicle = 0·028, germinal spot = 0·0085 millim.), and a granular yelk-mass has accumulated in them. They are contained in the genital tube, which measures about 3 millims. and is strongly folded together, and have not yet passed into the uterus, which is still short, and are destitute of shells. Perfectly developed eggs with shells are first seen in individuals of 5 millims. When the length has reached 6 millims., the uterus, which has in the meanwhile considerably increased in size, not only contains numerous mature ova, but also ova in all stages of embryonic development, and even perfectly mature embryos.

It is, however, exceedingly remarkable that the immigrated worms always develope only into female individuals. Even the pregnant animals bred by me were always unaccompanied by males. As I have nowhere else met with male specimens of *A. nigrovenosa*, although I have examined many hundred females, and have never found a trace of semen in the sexual organs, I have no hesitation in regarding the lung-worm of the frog as a *parthenogenetic creature*. That this is the first case of the kind among the Nematoda, or indeed among the Entozoa in general, can no more invalidate my supposition than the fact that older writers occasionally mention the male of *Ascaris nigrovenosa*. What they (Zeder, &c.) state about these so-called males by no means proves their existence, and leaves room for the supposition that younger specimens have been regarded as males.

How widely the developmental processes here described for *A. nigrovenosa* may be diffused among the Nematoda can only be ascertained by further investigations. At any rate, it would be too precipitate, starting from the preceding facts, to regard all the numerous *Rhabditis*-forms of mud and dung as mere developmental stages of parasitic roundworms. I am acquainted with nearly thirty different species of this group, but have not met with a single one which I could with any probability bring into the developmental cycle of a parasite.

However, I am not the first to assume alternating generations of parasitic and free forms for certain Nematoda. About ten years ago, an excellent naturalist, Carter, of Bombay, asserted that the embryos of the notorious *Filaria medinensis* did not become developed into parasites, but into free-living Rhabditiform worms (*Urolabes*, Carter), which grow to sexual maturity in water, and only resume a parasitic existence in their progeny. That doubt should have been repeatedly thrown upon this statement is perfectly explicable, considering the want of all objective foundation: even now it can only be regarded as questionable.

I know the embryos of *Filaria medinensis* from my own examinations, and can assert that they furnish no point of support for Carter's hypothesis. They are especially destitute of that peculiar development of the rudimentary sexual organs which appears so strikingly in the embryos of *Ascaris nigrovenosa,* and as it were foreshadows the remarkable destiny of this worm. To this may be added that the embryos of *Filaria* have been kept alive in water for a considerable time without change, by different observers.

If I were to put forward a supposition with regard to the destiny of these embryos, it would be to the effect that they are destined to an active immigration. I found this especially upon the considerable development of the tail, and the similarity which they present to the embryos of *Cucullanus.* The latter goes so far that one might easily be led to confound the two forms with one another, although the proportionate sizes do not exactly coincide. But it must be left to the future to decide whether it is the definitive host into which the embryos penetrate (through the sudoriferous pores, as Carter supposes), or an intermediate host, which is then probably introduced into the stomach. Indeed there are numerous surgeons who, in spite of all apparent reasons to the contrary, suppose the *Filaria medinensis* to penetrate into the muscles from the intestine.

On the other hand, and from the analogy of *Ascaris nigrovenosa,* I think I may accede to Bastian's supposition, *that the embryonal development of* Filaria medinensis *takes place without the concurrence of male individuals.* Hitherto no male example of this parasite has ever been found; and yet, although it is no doubt smaller than the female (considering the enormous fertility of the latter), it could hardly be overlooked on account of its size. The same consideration also excludes the supposition that the *Filaria,* although its size when seeking its definitive dwelling-place will be but small, has already received its store of seminal fluid.

Unfortunately I must here admit that my investigations upon the Nematoda of man present many other gaps. And yet, of course, the fate of these parasites is of the greatest importance to us. I can, however, add something even about these animals.

I may state, in the first place, that most of the Nematoda of the human subject belong to the species with hard and firm egg-shells. This applies especially to the more abundant species, *Trichocephalus dispar, Ascaris lumbricoides,* and *Oxyuris vermicularis.* The ova of the last-mentioned worm contain an embryo at the period of their escape from the female organs;

and this was probably in general overlooked (up to the time of Claparède) only in consequence of its peculiar form. It consists of a short and plump oval body (0·049 millim. in length, and 0·022 millim. in breadth), which occupies almost the whole space of the ovum, and of a thin conical tail (of 0·034 millim. in length) which is bent up on one side. Slender embryos, coiled up and moving briskly, such as occur in most Nematoda, and were ascribed by Vix also to *Oxyuris vermicularis,* have never been met with by me.

The ova of *Ascaris lumbricoides* and *Trichocephalus dispar* are only developed after a long sojourn in water or damp earth. (I have never been able to bring this development to completion in urine and in artificially prepared pits). In the first-mentioned worm the separation of the embryo usually requires from four to six months in summer, and in the other perhaps six to eight months; but the periods frequently oscillate in one or the other direction. Under certain conditions, the ova of *Ascaris lumbricoides* are not developed until the lapse of more than a year.

The embryo of *Ascaris lumbricoides* is much more slender than that of *Trichocephalus,* and is furnished with a short pointed tail, and a small tooth-like projection on the ventral side of the buccal orifice. It measures 0·25–0·28 millim., while the embryo of *Trichocephalus affinis,* which is rather larger than that of *T. dispar,* with the same thickness (0·01 millim.), only measures 0·127 millim. The embryos of *Ascaris mystax* and *A. marginata,* which inhabit the intestines of the cat and dog, and also require a period of several months for their incubation, although generally a shorter time than those of *A. lumbricoides,* present precisely the same characters, and only differ in size, the length in *A. mystax* being 0·38 millim., and in *A. marginata* even 0·42 millim. As, moreover, the egg-shells of the Nematode worms just mentioned possess the same thickness and resistant power, we may suppose that the fate of the young brood agrees with that of *A. lumbricoides.*

From the observations on other Nematoda above described, it might perhaps be anticipated that these embryos slip out of their shells when their development is completed. The shells are certainly thicker and firmer than in *Dochmius,* for example; but nevertheless such an assumption cannot be regarded *à priori* as erroneous—and this least of all with respect to *Trichocephalus,* the egg-shells of which bear an orifice at each pole, closed by a soft, nearly albuminous substance, like a stopper, which might easily be removed by the pressure of the worm. In *Ascaris,* indeed, these orifices are wanting; but, to make up for this, the embryos bear a dentary apparatus at the anterior end of the body (like that of *Ascaris acus*), and this might certainly aid them in breaking out of the egg-envelopes. Moreover it is not difficult to ascertain that the firmness of the egg-shells considerably diminishes in course of time.

Nevertheless I have not been able to effect the exclusion of the embryos of *Trichocephalus* and *Ascaris*, either in water or in moist earth. Even the addition of putrefying substances (such as fruit, potatoes, and beet) produced no result. It is true that during the investigation (especially with *Ascaris*) we sometimes find a few ruptured egg-shells, and even free embryos, but always in such small quantity that we remain in doubt whether their exclusion has been effected by the mechanical treatment of the objects or by the activity of the embryos. By far the greater part of the embryos remain within the egg-shells, and die there after a longer or shorter period.

The duration of life in the embryos seems to be longest in the species of *Ascaris*, which may not unfrequently be observed lively and mobile in their envelopes after the lapse of a year. Davaine states that he saw a portion of the embryos alive even in ova of five years old. In my experiments, however, decomposition commenced sooner, especially in the earth, where only a few living embryos could be detected after the lapse of fourteen months.

The embryos of *Oxyuris vermicularis* died within a few days, without any change of form, whether the ova were kept in water or in damp earth*.

Of course the only thing that remains to be done is to employ the mature ova with living embryos for the purpose of experiment. Starting from the assumption, already repeatedly expressed, that these ova could become developed at once in the ultimate parasite-bearer into the definitive worm, I made experiments in their administration several years ago. For this purpose I chiefly employed *Ascaris marginata*. The result was always negative. The dogs, which were killed at from six to twenty-one days after the administration, certainly often contained *A. marginata*, but always under circumstances which excluded all notion of their having possibly originated from the germs administered. I had no better success with *A. mystax* and *A. megalocephala*, or with *A. lumbricoides*, the ova of which were repeatedly administered to children by a surgeon of my acquaintance, and were twice swallowed in large quantities by adults.

Notwithstanding these constantly negative results, certain observations on the occurrence of the *Ascarides* led me to resume

* This applies also to the ova of *Oxyuris ambigua* of the Rabbit, which are deposited at the commencement of segmentation, and cannot be brought to any further stage of development.

these experiments at different times. In young dogs which were still sucking at their mother I repeatedly found these worms of from 1 to 2 inches in length, which must consequently to all appearance have been living as parasites for several weeks; on the other hand, I sometimes observed, in dogs which had been shut up for a long time, and fed upon broken victuals, worms measuring only a few millimètres, which therefore could not long have immigrated. The idea of the probability of a transfer in the egg-state seemed the more admissible in the latter case, because my dog-kennel was infected by previous experimental animals, so that, on microscopic examination, numerous ova of *Ascaris,* in earlier and later stages of evolution, were found on its floor. In one of these dogs, some of the *Ascarides* of which were only 3–4 millims. in length, a few ova with developed embryos were likewise to be found in the stomach.

In the presence of such observations, the assumption of an infection by means of mature ova must acquire more and more probability. It is true that experiments in this direction have hitherto given only negative results; but was it quite impossible that these were governed by certain individual and temporary peculiarities (age, nature of the stomachal fluids, &c.) of the experimental animal?

Taking these circumstances into consideration, the new experiments were made under varying conditions. Not only were dogs (and cats) of various ages employed, but these were subjected to experiment sometimes with a full, sometimes with an empty stomach, at a longer or shorter period after feeding.

Unfortunately the result in all these cases was equally negative with those of the previous experiments; and yet the number of experimental animals was not less than ten; moreover large quantities of ova were always administered, in some cases repeatedly at longer or shorter intervals. The examination was usually made soon after the last administration, sometimes only from six to twelve hours. In such cases there were usually still in the stomach numerous fragments of egg-shells, and even a few ova with a clear chorion and disintegrated (or at least decolorized) contents; but neither a living embryo nor a young *Ascaris* was ever found either in the intestine or in any other organ.

I must admit that it is with difficulty and unwillingness that I have given up the notion which served as the foundation of the above-mentioned experiments. By the proof that the ova furnished with mature embryos were developed directly into *Ascarides* in the intestine of man and the higher animals, the mode of occurrence of these worms with all its peculiarities would have been both simply and easily explained. But the facts were too clear and too accordant to permit one any longer to imagine an infection of this kind. If we had to do with matters of rare occurrence, we might certainly at least suppose that the circumstances of the experiment had not fulfilled the conditions necessary for the development of the parasites; but the worms in question are, as is well known, so abundant, that this objection can hardly be maintained.

That, however, there are really Nematoda which *migrate directly into their definitive host by the medium of ova containing embryos,* in the manner here indicated, is indubitable, from the results of other experiments made by me. The proof of such a development was most strikingly obtained in the case of *Trichocephalus affinis* of the sheep, the destiny of which, considering the perfect agreement of the ova and embryos, must be regarded as furnishing a rule for the *T. dispar* of man.

The embryos of this animal have already been mentioned as short plump worms, of 0·127 millim. They have a thick and a thin end, and terminal orifices to the alimentary canal, like the embryos of *Trichina,* but unlike those of all other Nematoda. They also resemble the embryos of *Trichina* in the fact that their organization exhibits but little differentiation. Their movements consist of very slow changes of position within the egg, during which the thicker end of the body is usually pushed in advance.

My material for experiment consisted of the whole of the ova of some twenty female individuals, which had been kept for about seven months in water, and had been completely decomposed therein. Sixteen days after administration, the experimental animal (a young lamb) was killed. To the naked eye the large intestine presented nothing unusual, but the microscope immediately revealed upon it many hundreds of young *Trichocephali.* The majority of these worms measured about 0·8–1 millim.; but there were specimens of only 0·5, and others of nearly 2 (one even of 2·4) millims. in length. Leaving out of consideration the absence of sexual development, they presented exactly the aspect of the intestinal *Trichinæ.* They were clear capilliform filaments (0·024 millim. thick), which, notwithstanding the presence of a diminished anterior end, showed as yet no indication of the characteristic ultimate form. In the interior, besides the cellular body, which traversed nearly the whole cavity of the body, the œsophagus and the short chyle-stomach were to be distinguished, the latter with a muscular terminal piece which, in the larger specimens (in the males), was not unfrequently clearly divided from the true stomach in the form of a distinct section. In the larger worms the genital tube could already be distinguished, running down beside the chyle-stomach.

The result of this experiment is so precise and convincing, that the above statement requires no further proof. But for this very reason it seems to me scarcely to be any longer doubtful that the *Ascarides* are developed in some other way, different from that of *Trichocephalus*. But if the embryos of these animals neither escape of themselves from their shells, nor pass with the shells into their definitive bearer, there hardly remains any other course except the assumption of an intermediate host.

But where are we to find this intermediate host?

I have administered the ova of *Ascaris lumbricoides* to a mouse without any result whatever. They passed out again undigested and with the embryos still living. I was led to make this experiment not by any hope of seeing the embryos developed into an intermediate form in the muscles of the mouse (for man will hardly introduce his roundworms by feeding on mice), but by the statement of Davaine that the embryos of *A. lumbricoides* fell out of their egg-capsules in great quantities in the intestines of the rat. It might perhaps be that the young worms only became capable of development when they had passed through the intestine of another animal and by this means had lost their shells. But here, again, I got a negative result.

The attempt to bring certain of the widely diffused lower animals to take up the ova of our *Ascarides* was equally unsuccessful. Earthworms, woodlice, and *Tenebriones*, which I kept in earth mixed with an abundance of the ova of *Ascaris lumbricoides*, never presented these ova or their embryos when dissected. The only animal which took them (and this was from water) was the *Asellus aquaticus*; but it yielded them in as unaltered a state as the mouse.

As, therefore, all my experiments left me just where I was before, I thought it necessary to strike into a new course. I put a number of young cats to live in a place from which I had repeatedly obtained animals with numerous young specimens of *Ascaris mystax*. This was a house outside the gates, with dunghills and kitchen-gardens in which the animals ran about freely and without any particular care being taken of them. After residing there for six or eight days, the animals were caught (usually in the morning), killed, and submitted to examination.

I had the satisfaction, in this way, not only of repeatedly detecting *Ascarides* of no great size (4–8 millims.), but also of making a discovery which, if it does not completely solve the question of their mode of importation, at least throws much light upon the destiny of our animals.

This related to a cat about eight weeks old, which had remained for six days in the above-mentioned place. The stomach and small intestine of the animal had collapsed, and contained no chyle; but the former contained a few bitten fragments of straw and all sorts of vegetable débris, among which a microscopic investigation distinctly showed fragments of potatoes and of potato-parings.

But the same stomach contained also at least forty to sixty Nematode embryos, some of which measured only 0·4–0·6 millim.; so that the smallest individuals were scarcely larger than the embryos of *Ascaris mystax* while still enclosed in their egg-capsules. However, there could not be the least doubt that the embryos which I found here free and twisting about briskly on the mucous membrane of the stomach of the young cat were those of *Ascaris mystax*. Not only did they, especially the smallest specimens, agree exactly with the animals from the eggs with which I was so well acquainted, but I could also follow them through all stages of development up to young *Ascarides* of 3–4 millims. in length, which occurred, together with the larger embryos, in the small intestine, and, notwithstanding their small size and slender form, already presented precisely the characters of the *Ascaris mystax* of the cat.

In the first place it was proved by this discovery that *Ascaris mystax* (and also decidedly the other allied *Ascarides*, including therefore *A. lumbricoides*) retains its original developmental condition up to the time of its introduction into its definitive host, or, in other words, *immigrates into its definitive bearer in the embryonic form*. In this respect *A. mystax* therefore behaves like the above-mentioned *A. acus*; nay, it even surpasses the latter, as before its transfer to its definitive habitat it does not even increase in size.

But what the embryos previously wanted is made up immediately after their immigration. The embryos grow, without, however, essentially altering their structure; they grow rapidly, and pass, when about 1·5–2 millims. in length, from the stomach into the small intestine. The intestinal cells gradually acquire a brownish colour during the increase of size. The muscular sac is thickened, and the glandular stomach gradually separates from the posterior extremity of the œsophagus as a special structure. But the rudimentary sexual organ still remains without any further development, and the mouth still bears the embryonic boring-tooth instead of the three lips. These conditions are altered only when the next change of skin takes place, at a length of 2·8 millims. A little while before this, the genuine structure of the Ascaride mouth may be distinguished under the cuticle of the head; and the increase of the genital rudiment into a short sac, either simple or Y-shaped, according to the sex, may be observed. The formation of the spicula only takes place subsequently, when the worm has already attained a length of 10–12 millims., and long been furnished with the wing-like cephalic ridges (which make their appearance when it is about 6 millims. in length).

Unfortunately this interesting discovery has given us no definite information as to the mode of introduction. No remains of animal matters could be detected in the contents of the stomach; but who can tell how long the embryos had already been in the stomach? A second young cat, which had eaten the mucous membrane of the stomach of the former, together with the parasites still living in it, showed the worms on the following day likewise in the stomach, and hardly perceptibly altered from their previous condition.

It appears clear to me, however, that it is not by any of the larger animals that the embryos of *Ascarides* are conveyed into the intestine of their definitive bearer. As things remain from the preceding observations, we need for the completion of our knowledge of the life-history of the *Ascarides* only a single element. May we soon succeed in filling up this gap, and thus bring the commonest of the human Entozoa completely within the domain of science.

L.—*Note on some new Facts in Botanical Geography.* By Edmond Boissier*.

By generalizing observations which are nearly always incomplete (as we are still but imperfectly acquainted with most floras), botanists, ascertaining the predominance in some particular botanical region of certain families or genera, hurry sometimes, and prematurely, to the conclusion that this region is their exclusive habitat. Nevertheless new facts come from time to time to show us that there is nothing absolute in the laws which have governed the present distribution of plants upon the surface of the globe; and some interest attaches to the registration of these facts and to the combination in this manner of the materials which will perhaps hereafter assist in explaining the formation of the different floras.

There have recently been discovered in Europe and Asia Minor some species which are particularly interesting, inasmuch as their congeners inhabit very distant regions. The first of these is a *Dioscorea*. The *Dioscoreæ* are diœcious monocotyledonous plants with generally a twisting and climbing stem. Their root is a tuber; and that of some species is employed as food, under the name of Yam. The genus *Dioscorea* is very numerous in

* From the 'Bibliothèque Universelle,' March 25, 1866, Archives des Sciences, pp. 255–260.